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(54) Injectable composition for the treatment of helminthiasis and clostridial diseases in animals

(57) Injectable compositions comprise tetramisole or its laevorotatory isomer and a vaccine preferably Clostridial vaccine or mixture of (i.e. a multivalent) Clostridial vaccines and are used for the treatment of sheep and cattle to combat helminthiasis and Clostridial diseases.

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SPECIFICATION

Injectable composition for the treatment of helminthiasis and clostridial diseases in animals

This invention relates to a new composition of matter comprising the imidazo[2,1-b]thiazole tetramisole and a vaccine and to processes for its use; in particular it relates to a stable composition suitable for administration by injection and to its use in the treatment of helminthiasis and Clostridial diseases in warm-blooded animals.

D,L-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole, hereinafter referred to as D,L-tetramisole, and its pharmaceutically acceptable acid addition salts are potent anthelmintic agents and the laevorotatory isomer, hereinafter referred to as L-tetramisole, is responsible for all or most of the anthelmintic activity, at least in ruminants such as sheep or cattle. One of the preferred methods for administration of tetramisole is by subcutaneous injection of an aqueous solution and our Australian Patents No 440,746 and 450,036 disclose aqueous formulations suitable for administration by injection.

Vaccines are widely used to protect warm-blooded animals from a wide range of diseases. Anaerobic vaccines have proved to be of considerable importance in the prevention of a number of common diseases of domestic animals such as cattle, sheep, pigs and fowl. Of particular importance have been anaerobic vaccines for the prevention of Clostridial diseases such as, for example, Pulpy Kidney (Enterotoxaemia), Blackleg, Malignant Oedema (blood poisoning), Tetanus and Black disease, in sheep and cattle.

Although in the past both tetramisole and vaccines have been administered to animals by subcutaneous injection they have always been administered separately because it was hitherto believed to be impossible to combine a vaccine and tetramisole in a stable aqueous formulation suitable for injection because of the different stability requirements for aqueous tetramisole formulation and vaccine formulations.

Tetramisole readily undergoes base-catalysed hydrolysis to an inactive derivative and as a result aqueous tetramisole solutions are adjusted to an acid pH to prevent loss of the active ingredient. The aqueous formulations disclosed in Australian Patents No 440,746 and 450,036 are adjusted to a pH of less than 4, preferably approximately 3.5, to provide the formulations with the required storage stability.

In contrast, it is well known that in order to maintain their activity vaccines should not be subjected to a pH of less than 6.0 or more than 7.0 and that as a general rule vaccines are unstable at low pH conditions which promote the denaturing of proteins. For example, J R Hepple in "International Symposium on Adjuvants of Immunity, Utreckt 1966; Symp. Series Immunobiol. Standard", Vol 6 pp. 173–180, Karger, Basel/New York 1967, reports that with Clostridial vaccines it is important to maintain the pH in the range 6.1 to 6.4. Too high a pH results in desorption of the antigen from the carrier while at low pH's denaturing of the antigens can occur, *Clostridium perfringens* type B and *Clostridium septicum* being sensitive to pH values below 6.0.

Furthermore, when formulated as an aqueous injectable solution tetramisole is preferably in the form of the hydrochloride, citrate, tartrate or more preferably the dihydrogen phosphate salt and may be accompanied by water soluble therapeutically acceptable salts, particularly the sodium or potassium salts of citric, tartaric or phosphoric acid, in order to prevent or reduce adverse tissue reaction at the site of injection. Vaccines on the other hand are in general incompatible with certain anions such as citrate, phosphate and sulfate because these anions can cause the elution of the antigen from the carrier adjuvant to which it is reversibly bound, thereby inactivating the vaccine or reducing its storage stability.

The abovementioned apparently incompatible formulation requirements for injectable compositions of tetramisole and vaccines has meant that tetramisole has not previously been combined with a vaccine in the one formulation suitable for administration to warm-blooded animals by subcutaneous injection. We have now surprisingly found that tetramisole and vaccines may be combined in one formulation suitable for subcutaneous injection without adversely affecting either the efficacy or the stability of the tetramisole or the vaccine.

Accordingly, the invention provides an acidic aqueous composition, which is therapeutically acceptable to warm blooded animals by injection, said composition comprising a tetramisole salt or a laevorotatory tetramisole salt and a vaccine.

Preferably the tetramisole is in the form of a salt of the laevorotatory isomer (L-tetramisole or levamisole).

Suitable L-tetramisole salts include the hydrochloride, acetate, citrate, tartrate and phosphate salts. Preferably the tetramisole is in the form of the L-tetramisole dihydrogen phosphate salt.

Suitable vaccines include anaerobic vaccines and in particular anaerobic vaccines for the prevention of Clostricial diseases in sheep and cattle. Suitable vaccines include the Clostridial vaccines described in the paper by J R Hepple referred to above and the references cited

therein. Such vaccines include, for example, those which contain antigens prepared from strains of Clostridia such as *Clostridium welchii* (*Clostridium perfringens*) types B, C and D), *Clostridium septicum*, *Clostridium tetani*, *Clostridium chauvoei* and *Clostridium novyi* (*Clostridium oedematiens*) type B which are used in the treatment of Lamb dysentery, Pulpy Kidney disease (enterotoxaemia), Malignant Oedema (blood poisoning), Tetanus, Blackleg disease and Black disease, and combinations of one or more of those antigens.

As hereinbefore discussed, aqueous tetramisole solutions are preferable adjusted to an acid pH to prevent the hydrolysis of the tetramisole to an inactive derivative. Accordingly, the injectable compositions of the invention are preferably adjusted to an acid pH in the range from 2.0 to 6.0, and more preferably to a pH in the range from 3.0 to 4.0, by the addition of an acid having a therapeutically acceptable anion such as, for example, hydrochloric, tartaric, citric, or preferably phosphoric acid.

The injectable compositions of the invention may also comprise therapeutically acceptable salts such as, for example, the sodium salts of citric, tartaric or phosphoric acid or mixtures thereof in order to prevent or reduce the incidence of adverse tissue reaction. If present, the therapeutically acceptable salts are preferably at a concentration equivalent to from 0.1 to 0.15 moles per litre of solution.

Vaccines are normally prepared and stabilized in the presence of additives known as vaccine adjuvants. Thus the injectable compositions of the invention preferably comprise pharmaceutically acceptable adjuvants and/or preservatives including antigen carriers.

Suitable adjuvants include potassium alum, protamine, aluminium phosphate, aluminium hydroxide, calcium phosphate, glycerol, sorbitol, propylene glycol, carboxyvinyl polymers available under the Trade Mark "Carbopol" and bearing the designation 934, 940 and 941, Freund's universal adjuvant, soluble diethylaminoethyl (DEAE) dextran, saponin, "Quil-A", sodium chloride solution, and the fixed oils and synthetic esters of higher fatty acids which are known to be effective adjuvants.

Suitable preservatives include phenol, formaldehyde, propylene glycol, glycerol, esters of *p*-hydroxybenzoic acid, benzoic acid and its sodium salt, hexachlorophene, quaternary germicides and thiomersal as such or in the form in which it is available under the Trade Mark "Merthiolate".

In preparing the injectable compositions of the invention it has been found preferable to formulate the vaccine and tetramisole components separately in the normal manner and then to mix the two components and then to adjust the pH of the combined vaccine-tetramisole composition before storage and subsequent use. In contrast to all expectations it has been found that the injectable compositions of the invention prepared in this fashion and adjusted to an acid pH do not precipitate vaccine toxoids and the activity of both the vaccine component and the tetramisole component is maintained on storage.

In view of the known incompatible requirements for the formulation on the one hand of stable aqueous compositions of tetramisole and on the other hand stable vaccine compositions the present invention of a stable combined tetramisole-vaccine composition is completely unexpected. Moreover, it should be noted that the compositions of the invention are not merely stable for a short period of time after preparation. The efficacy of the compositions is unimpaired after long storage under the conditions normally employed to store vaccines.

The injectable compositions of the invention comprising a (L-) tetramisole salt and a Clostridial vaccine have been found to be effective in killing helminths in warm-blooded animals and in vaccinating said animals against Clostridial diseases when administered parenterally to said animals. Accordingly in a further aspect the invention provides a process for combatting helminthiasis and Clostridial diseases in warm-blooded animals by the parenteral administration of a therapeutically effective amount of a composition comprising a tetramisole salt or a laevorotatory tetramisole salt and a Clostridial vaccine.

The term "parenteral" is used herein to mean intravenous, intramuscular and subcutaneous injection. Preferably the compositions are administered according to the process of the invention by subcutaneous injection.

The process of the invention is particularly suitable for the treatment of large farm animals such as sheep and cattle. However, the process may also be used to treat helminthiasis and Clostridial diseases in other domestic, farm and laboratory animals.

It will be evident to those skilled in the art that the process of the invention offers the advantage of being able to treat a warm-blooded animal with an anthelmintic and a vaccine in the one operation with important savings in labour costs. This advantage may be put to particular benefit in the treatment of pregnant ewes before lambing. In the past, the conventional procedure has been to treat pregnant ewes with an anthelmintic 4 to 6 weeks before lambing and then to treat the ewes with a Clostridial vaccine 2 weeks before lambing. It has now been found that these two operations can be combined by treating pregnant ewes with a composition of the invention comprising a (L-) tetramisole salt and a Clostridial vaccine 4 to 6 weeks before lambing.

When formulated into a composition of the invention no loss in activity has been observed in either the tetramisole component or the vaccine component. Therefore, the compositions are preferably formulated to contain, in a suitable dosage volume, the dose of (L-) tetramisole and the dose of vaccine usually employed in the treatment of that particular animal when the (L-) tetramisole and the vaccine are parenterally administered separately, as single therapeutic agents.

Such dose rates vary with the animal being treated and the specific (L-) tetramisole salt and vaccine being used. However, in general L-tetramisole is administered at a dose rate of approximately 5 to 10 mg (calculated as the free base) per kilogram of animal bodyweight, D,L-tetramisole is administered at a dose rate of approximately 10 to 17 mg (calculated as the free base) per kilogram of animal body weight and in general vaccine preparations have been standardized to a dose volume of 2 ml for sheep and 4 ml for cattle for mono-, di-, tri- tetra- and multivalent vaccines.

In combatting diseases by vaccination it is usual to administer two doses of vaccine the second dose being administered at least four weeks after the first dose. Thus in order to optimize the protection afforded by the vaccine component of the composition of the invention it is preferable to repeat the parenteral administration of a therapeutcially effective amount of the composition at least four weeks later.

The compositions of the invention may comprise, in addition to the components hereinbefore defined: other pharmaceutically therapeutic agents such as, for example, flukicides, selenium (to combat white muscle disease) and systemically active pesticides; additives to improve the shelf life of the composition; buffering agents; preservatives; and/or additives to prevent or to reduce adverse tissue reaction at the site of the injection.

The invention is now illustrated by, but by no means limited to, the following Examples.

Example 1

In order to evaluate the stability and the efficacy of the tetramisole-clostridial vaccine compositions of the invention injectable compositions A_1, A_2 and A_3 were prepared by admixture of a 7 component Clostridial vaccine comprising antigens prepared from *Clostridium welchii* Type B, *Clostridium welchii* Type C, *Clostridium welchii*, Type D, *Clostridium septicum*, *Clostridium tetani*, *Clostridium chauvoei* and *Clostridium novyi* Type B [available from ICI Tasman Limited under the name "Tasvax" 7 ("Tasvax" is a Trade Mark)] and an aqueous sterile filtered solution of L-tetramisole dihydrogen phosphate (17.6% w/v free base) and adjustment of the pH of the composition to the required level before storage and subsequent use. The make up of the test compositions and the control compositions are detailed in Table 1 below:

TABLE 1

Composition+ No	Vaccine Component Volume (ml); pH		Tetramisole Component Volume (ml); pH		pH of Composition
A_1	500;	3.75	425;	3.5	3.55
A_2	500;	5.35	425;	5.0	5.3
A_3	500	6.05	425;	5.9	6.0
Control 1	500	6.3	—	—	—
Control 2	—	—	500*;	3.45	—

+ After formulation each composition was stored at 4° to 8°C in a glass bottle
* L-tetramisole dihydrogen phosphate of free base concentration 6.8% w/v.

Example 2

Each of the compositions and control compositions detailed in Table 1 above was tested for efficacy by injection into 2 sheep following the dosing schedule detailed in Table 2 below.

TABLE 2

Day	Operation	
0	Compositions formulated	
	(a) Sheep tagged; Blood serum sample taken; Faecal egg count made	
	(b) Sheep injected as follows:	
	Composition A_1	3.5 ml
	Composition A_2	3.5 ml
	Composition A_3	3.5 ml
	Control composition 1	2.0 ml
	Control composition 2	4.0 ml
4	Faecal egg count made	
43	(a) Faecal egg count made	
	(b) Injections given on Day 1 repeated	
46	Faecal egg count made	
57	Blood serum sample taken	

The anthelmintic activity of the test compositions was evaluated by measuring the faecal egg count of the sheep before and after treatment and the results are presented in Table 3 below. It should be noted that the sheep were reinfected by helminths between treatments.

TABLE 3

Test Composition	Faecal Egg Count			
	Day 1	Day 4	Day 43	Day 46
A_1	500	0	1200	0
A_1	200	0	1600	0
A_2	350	0	1100	0
A_2	100	0	800	0
A_3	50	0	1900	0
A_3	150	0	3350	0
Control 1	100	200	1350	700
Control 1	750	1100	850	1100
Control 2	750	0	900	0
Control 2	350	0	2450	0

The antigenicity of the vaccines was evaluated by assaying the blood serum samples taken at day 57 for *Clostridium perfringens* Type C (*Clostridium welchii* Type C; common name–lamb dysentery); *Clostridium perfringens* Type D (*Clostridium welchii* Type D; common name–pulpy kidney); *Clostridium novyi* Type B (*Clostridium oedematiens* Type B; common name–Black disease) and *Clostridium tetani* (common name–tetanus) antitoxins using conventional assay methods using mice. The results are presented in Table 4 below.

TABLE 4

Test Compositions	Antitoxin Titre+ (units/ml) LD	PK	BD	TET
A_1	>27	13–16	>13.5	>13.5
A_1	13–27	32–40	8–13.5	8–13
A_2	13–27	13–16	>13.5	>13
A_2	<5	3.2	3–8	<3
A_3	13–27	20–28	8–13	3–8
A_3	5–13	6.6	3–8	3–8
Control 1	<5	20–27	8–13.5	8
Control 1	5–13	20–27	8–13.5	8–13
Control 2	<5	<0.7	<3.3	—
Control 2	<5	2–3	<3.3	—

+ LD–lamb dysentery
PK–pulpy kidney
BD–Black disease
TET–tetanus

Example 3

After storage for 6 months at a temperature of 4°C the compositions A_1 and A_3 and control compositions Control 1 (C_1) and Control 2(C_2), prepared as detailed in Example 1, were tested for efficacy by injection into sheep following the dosing schedule detailed in Table 5 below. Each composition was tested on 40 sheep with a control group of six sheep not being treated.

TABLE 5

Day	Operation
1	(a) Blood serum sample taken
	(b) Group A_1 sheep injected with 3.5 ml of Composition A_1 Group A_3 sheep injected with 3.5 ml of Composition A_3 Group C_1 sheep injected with 2.0 ml of Composition C_1 Group C_2 sheep injected with 4.0 ml of Composition C_2
42	(a) Blood serum sample taken
	(b) Injections given on Day 1 were repeated
56	Blood serum sample taken
98	Blood serum sample taken

The antigenicity of the vaccines was evaluated by assaying the blood serum samples taken at days 56 and 98 for *Clostridium perfringens* Type D (*Clostridium welchii* Type D; common name–pulpy kidney), *Clostridium septicum* (common name–malignant oedemia), *Clostridium novvi* Type B (*Clostridium oedematiens* Type B; common name–Black disease), *Clostridium tetani* (common name–tetanus) and *Clostridium perfringens* Type C (*Clostridium welchii* Type C; common name–lamb dysentery). The antitoxin titres on the pooled serum samples from the 40 sheep in each group were determined by conventional assay methods using mice. The results are presented in Table 6.

TABLE 6

Test Composition	Day of Bleed	Antitoxin Titre+ (units/ml) PK	MO	BD	TET	LD
A_1	56	16–20	13.3–16	27–32	20–26.7	26–32
A_1	98	3.2–4.0	2–4	4–5.3	3.2–4	2–4
A_3	56	6.65–8.0	5.3	10–13.3	13.3–16	10–13.3
A_3	98	1–2	0.67–1.0	1–2	1–2	0.67
C_1	56	5–6.65	4	20–27	13.3	8–10
C_1	98	0.67–1.0	0.67–1.0	2	1–2	0.67–1.0
C_2	56	<0.67	<0.67	<0.67	<0.67	<0.67
Untreated	56	<0.67	<0.67	<0.67	<0.67	<0.67

+ PK–pulpy kidney
MO–malignant oedemia
BD–Black disease
TET–tetanus
LD–lamb dysentery

The only meaningful test available to determine the effectiveness of the vaccination against *Clostridium chauvoei* (blackleg) is by direct challenge with a living culture or spore suspension. After completion of the dosing and bleeding schedule in Table 5 above five sheep from each of the groups injected with Test Compositions A_1 and C_1 were injected intramuscularly with 2 ml of an 18 hour culture of *Clostridium chauvoei* (strain F6028) containing 2.5% calcium chloride solution. Two of the untreated controls were injected with one tenth, and one of the untreated controls was injected with one hundredth, of the dose injected into the vaccinated sheep. Within 24 hours of the challenge with *Clostridium chauvoei* the three unvaccinated controls had died while the ten vaccinated sheep survived the direct challenge.

Example 4

This example demonstrates the preparation of compositions of the invention comprising a 5 component Clostridial vaccine and L-tetramisole dihydrogen phosphate.

A pentavalent Clostridial vaccine (Control 3) comprising antigens prepared from *Clostridium welchii* type D, *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium oedematiens* and *Clostridium tetani* (500 parts containing 0.53 standard dose units per part) was combined with an aqueous solution of L-tetramisole dihydrogen phosphate (400 parts containing 15.3% w/v L-tetramisole calculated as the free base) and the pH of the resulting composition was adjusted to 3.5 by the addition of phosphoric acid. The composition (code number A_4) contained 0.29 standard dose units of vaccine per ml and 68 mg per ml of L-tetramisole (calculated as the free base).

The above procedure was repeated by combining the pentavalent Clostridial vaccine (Control 3; 500 parts containing 0.53 standard dose units per ml) with an aqueous solution of L-tetramisole dihydrogen phosphate (350 parts containing 18.2% w/v L-tetramisole calculated as the free base) and the pH of the resulting composition was adjusted to 3.5 by the addition of phosphoric acid. The composition (code number A_5) contained 0.31 standard dose unit of vaccine per ml and 75 mg/ml of L-tetramisole (calculated as the free base).

The above procedure was repeated by combining the pentavalent Clostridial vaccine (Control 3; 500 parts containing 0.53 standard dose units per ml) with an aqueous solution of L-tetramisole dihydrogen phosphate (560 parts containing 11.4% w/v L-tetramisole calculated as the free base) and the pH of the solution was adjusted to 3.5 by the addition of phosphoric acid. The composition (code number A_6) contained 0.25 standard dose units of vaccine per ml and 60.2 mg/ml of L-tetramisole (calculated as the free base).

Example 5

This Example demonstrates the preparation of a composition of the invention comprising a 5 component Clostridial vaccine and the hydrochloride salt of L-tetramisole.

A pentavalent Clostridial vaccine (Control 3) comprising antigens prepared from *Clostridium welchii* type D, *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium oedematiens* and *Clostridium tetani* (500 parts containing 0.53 standard dose units per part) was combined with an aqueous solution of the hydrochloride salt of L-tetramisole (560 parts containing 11.4% w/v L-tetramisole calculated as the free base) and the pH of the solution was adjusted to 3.5 by the addition of hydrochloric acid. The composition (code number A_7) contained 0.25 standard dose

units of vaccine per ml and 60.2 mg/ml of L-tetramisole (calculated as the free base).

Example 6

This Example demonstrates the antigenic efficacy of the compositions of the invention after prolonged storage.

Immediately after preparation the antigenicity of composition A_1, prepared as described in Example 1, was tested in laboratory rabbits (Test No 1). Each rabbit was injected on day 0 with 2.0 ml of composition A_1 and the injection was repeated 42 days later. After 2 weeks (day 56) a blood sample was taken from each rabbit and the antitoxin titres of the pooled samples were determined by conventional assay methods.

After storage for a period of 9 months at a temperature of approximately 4°C the antigenicity of composition A_1 was retested (Test No 2) in laboratory rabbits following the procedure described above with the exception that the amount of compositon A_1 injected on each of days 0 and 42 was reduced from 2.0 to 1.4 ml (30% reduction in dose).

The results are presented in Table 7, the code for the antitoxins assayed being the same as that used in Example 3 Table 6.

TABLE 7

Test No	Dose Size	Antitoxin Titre (units/ml)				
		PK	MO	BD	TET	LD
1	2 × 2 ml	6.6	6.4–8.0	5.3–6.4	6.6–8.0	20–27
2	2 × 1.4 ml	5–6.6	5–6.6	4–5	6.6	6.6–8.0

Example 7

This Example demonstrates the anthelmintic efficacy of the compositions of the invention.

To ensure that the compositions were tested in heavily infected animals Merino weaners harbouring a heavy naturally acquired parasitic infection were chosen and further infected with larvae of the species *Ostertagia* and *Trichostrongylus*. The nematode infections were allowed to reach maturity and the sheep were divided into four groups of eight animals each, the division being made on the basis of faecal egg count to ensure that the groups had a similar mean infection. The sheep were weighed and treated, on the basis of their weight, with sufficient test composition to ensure a dose rate of 6.0 mg per kg of animal body weight of L-tetramisole base. The four groups were treated as follows:

Group 1—Control (no treatment)
Group 2—L-tetramisole dihydrogen phosphate (6.0% w/v base)
Group 3—Composition A_4 (Example 4)
Group 4—Composition A_5 (Example 4) which had been stored for 6 months at a temperature of approximately 4°C.

The compositions were administered by subcutaneous injection into the neck. The animals were slaughtered 4 to 5 days after treatment and the paraistes in the abomasum, small intestine, large intestine and lungs were counted.

The total and the mean parasite counts for each Group are presented in Table 8 in which the parasites are coded as follows:

H — *Haemonchus*
O — *Ostertagia*
TA — *Trichostrongylus axei*
I — Immature parasites
TR — *Trichostrongylus spp*
N — *Nematodirus spp*
C — *Cooperia oncophora*
CH — *Chabertia*
OE — *Oesphogostomum venulosum*
D — *Dictyocaulus* (lungworm)

TABLE 8a
Parasite Count—Abomasum

Group	Count	Parasite H	O	TA	I
Group 1	Total	1260	26070	16100	9500
	Mean	157	3260	2012	1187
	% Efficiency	—	—	—	—
Group 2	Total	0	390	170	1100
	Mean	0	98	21	137
	% Efficiency	100	97.1	99.9	88.5
Group 3	Total	0	750	360	950
	Mean	0	94	45	119
	% Efficiency	100	97.2	97.8	90.0
Group 4	Total	0	800	330	300
	Mean	0	100	41	37
	% Efficiency	100	97.0	98.0	96.9

Table 8b
Parasite Count—Small Intestine

Group	Count	Parasite TR	N	C
Group 1	Total	31600	10360	19960
	Mean	3950	1295	2495
	% Efficiency	—	—	—
Group 2	Total	30	0	0
	Mean	4	0	0
	% Efficiency	99.9	100	100
Group 3	Total	30	110	0
	Mean	4	14	0
	% Efficiency	99.9	99.9	100
Group 4	Total	40	40	0
	Mean	5	5	0
	% Efficiency	99.9	99.6	100

TABLE 8c
Parasite Count—Large Intestine and Lung

Group	Count	Parasite—Large Intestine CH	Parasite—Large Intestine OE	Parasite—Lung D
Group 1	Total	0	1364	70
	Mean	0	170	9
	% Efficiency	—	—	—
Group 2	Total	0	1	2
	Mean	0	0.12	0.25
	% Efficiency	—	99.9	97.3
Group 3	Total	0	6	0
	Mean	0	0.75	0
	% Efficiency	—	99.6	100
Group 4	Total	0	2	0
	Mean	0	0.25	0
	% Efficiency	—	99.9	100

Example 8

This Example demonstrates the antigenic efficacy of the compositions of the invention in cattle.

Three months old cattle which had not previously been vaccinated were selected and divided into four groups. The cattle in each group were treated on day 0 and again on day 28 by subcutaneous injection with composition A_6 (Example 4) or the standard pentavalent vaccine (Control 3) used in the preparation of composition A_6 (Example 4) as follows:

Group 1—Standard vaccine Control 3; dose 4.0 ml (normal cattle dose)
Group 2—Composition A_6; dose 4.0 ml (half normal cattle dose)
Group 3—Composition A_6; dose 8.0 ml (normal cattle dose)
Group 4—Composition A_6; dose 16.0 ml (twice normal cattle dose).

Two weeks after the final injection (day 42) a blood sample was taken from each of the animals and the antitoxin titres of the pooled sera samples from each Group were determined by conventional assay methods.

The results are presented in Table 9, the code for the antitoxins assayed being the same as that used in Example 3 Table 6.

TABLE 9

Group	Antitoxin Titre (units/ml) PK	MO	BD	TET
1	1.0	2.0	10–13	10
2	4–5.3	<0.67	6–8	8–10
3	8–10	1–2	20–26.7	20–26.7
4	10–13	2–4	26–32	53–64

CLAIMS

1. An acidic aqueous composition which is therapeutically acceptable to warm blooded animals by injection said composition comprising a tetramisole salt or a laevorotatory tetramisole salt and a vaccine.

2. A composition according to claim 1 wherein said vaccine is an anaerobic vaccine.

3. A composition according to claim 1 or claim 2 wherein said vaccine is a Clostridial vaccine.

4. A composition according to any one of claims 1 to 3 inclusive wherein said vaccine is a Clostridial vaccine which comprises antigens prepared from Clostridia chosen from the group *Clostridium welchii* type B, *Clostridium welchii* type C, *Clostridium welchii* type D, *Clostridium septicum*, *Clostridium tetani*, *Clostridium chauvoei* and *Clostridium novyi* type B or a combination of one or more of said antigens.

5. A composition according to any one of claims 1 to 4 inclusive wherein said tetramisole

salt or laevorotatory tetramisole salt is chosen from the hydrochloride, acetate, citrate, tartrate and phosphate salts of laevorotatory tetramisole.

6. A composition according to claim 5 wherein said salt is the hydrochloride salt of laevorotatory tetramisole.

7. A composition according to claim 5 wherein said salt is the dihydrogen phosphate salt of laevorotatory tetramisole.

8. A composition according to any one of claims 1 to 7 inclusive wherein the pH of said compostion is in the range from 2.0 to 8.0.

9. A composition according to any one of claims 1 to 8 inclusive wherein the pH of said composition is in the range from 3.0 to 4.0.

10. A composition according to any one of claims 1 to 9 inclusive having a pH in the range of from 3 to 4 and comprising the hydrochloride salt of L-tetramisole and vaccine antigens prepared from *Clostridium welchii* type B, *Clostridium welchii* type C, *Clostridium welchii* type D, *Clostridium septicum*, *Clostridium tetani*, *Clostridium chauvoei* and *Clostridium novyi* type B.

11. A composition according to any one of claims 1 to 9 inclusive having a pH in the range of from 3 to 4 and comprising the dihydrogenphosphate salt of L-tetramisole and vaccine antigens prepared from *Clostridium welchii* type B, *Clostridium welchii* type C, *Clostridium welchii* type D, *Clostridium septicum*, *Clostridium tetani*, *Clostridium chauvoei* and *Clostridium novyi* type B.

12. A composition according to any one of claims 1 to 11 inclusive comprising therapeutically acceptable salts chosen from the sodium salts of citric, tartaric and phosphoric acid or mixtures thereof at a concentration equivalent to from 0.1 to 0.15 moles per litre of solution.

13. A composition according to any one of claims 1 to 12 inclusive comprising pharmaceutically acceptable vaccine adjuvants and/or preservatives.

14. A composition according to any one of claims 1 to 13 comprising one or more additional medicinal or therapeutic agents.

15. A process for combatting helminthiasis and Clostridial diseases in warm-blooded animals which process comprises the parenteral administration of a therapeutically effective amount of a composition comprising a tetramisole salt or a laevorotatory tetramisole salt and a Clostridial vaccine as defined according to any one of claims 3 to 14 inclusive.

16. A process according to claim 15 wherein said warm-blooded animals are sheep.

17. A process according to claim 15 wherein said warm-blooded animals are cattle.

18. A process according to claim 16 wherein said composition is administered to pregnant ewes 4 to 6 weeks before lambing.

19. A process according to any one of claims 15 to 18 inclusive wherein said composition is administered by subcutaneous injection.

20. A composition according to any one of claims 1 to 14 inclusive substantially as herein described with reference to any one of Examples 1, 4 or 5.

21. A process according to any one of claims 15 to 19 inclusive substantially as herein described with reference to any one of Examples 2, 3 or 6 to 8 inclusive.

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